Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography A, 1097 (2005) 183–187
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Short communication

Simultaneous determination of carbamate and organophosphorus pesticides in fruits and vegetables by liquid chromatography–mass spectrometry

Min Liu [a], Yuki Hashi [b,*], Yuanyuan Song [b], Jin-Ming Lin [a,*]

[a] *State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing 100085, China*
[b] *Shimadzu (HongKong) Limited, Beijing Office, Analytical Applications Center 14F No. 16, Chao Yang Men Wai Street, Beijing China, Life Tower Chao Yang District, Beijing 100020, China*

Received 15 July 2005; received in revised form 8 October 2005; accepted 13 October 2005
Available online 27 October 2005

Abstract

A liquid chromatography–mass spectrometry (LC–MS) method was established for the purpose of simultaneous determination of carbamate and organophosphorus (OPPs) pesticides in fruits and vegetables. Samples were extracted with acetonitrile; and then prepared by dispersive solid-phase extraction (dispersive-SPE) with primary secondary amine (PSA) as the sorbent. Four common representative samples (tomato, apple, carrot, and cabbage) were selected from the supermarket to investigate the effect of different matrices on pesticides recoveries and assay precision after spiking samples with 0.05 mg/kg. Matrix composition did not interfere significantly with the determination of the pesticides. The obtained recoveries were, with a few exceptions, in the range of 70–110% with RSDs less than 8%. It was applied to pesticide residue monitoring in vegetables and fruits from local markets.

Keywords: Carbamates; Organophosphorus; Liquid chromatography–mass spectrometry; Dispersive solid-phase extraction

1. Introduction

Carbamates and organophosphorus (OPPs) pesticides are the most widely used pesticides. Therefore, it has been necessary to develop accurate analytical method for the identification and quantification of OPPs and carbamate pesticides in fruits and vegetables. Due to their physical chemical properties such as thermal instability and polarity, carbamates are difficult or even impossible to be analyzed using GC techniques without the time-consuming process of derivatization. Due to lower sensitivity of LC/UV, HPLC with fluorescence detection by post-column derivatization is the most widely used method for the analysis of carbamate pesticides in foods [1]. Most OPPs are easily analyzed by GC. Therefore, carbamates and OPPs are usually analyzed by liquid chromatography and gas chromatography, respectively. Just so, the procedure is time-consuming.

LC–MS has now emerged as an excellent alternative technique for simultaneous analysis of these compounds. Several reports attempted to use LC–MS methods for the simultaneous determination of carbamates and organophosphorus pesticides in vegetables and fruits [2–6]. However, these works were mainly based on traditional extraction with organic solvent. It is simple yet time-consuming and consumes much toxic solvents as well [2–6].

Anastassiades et al. [7] firstly established dispersive-SPE for the determination of pesticides in vegetables and fruits by GC/MS. Lehotay et al. [8–10] developed the method to analyze pesticides by gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/tandem mass spectrometry (LC–MS/MS). Posyniak et al. [11] recently applied the procedure to analyze sulfonamides in chicken by liquid chromatography with fluorescence detection. LC–MS with a single quadrupole has also been widely reported to deter-

* Corresponding authors. Tel.: +86 10 62841953; fax: +86 10 62841953.
E-mail addresses: y-hashi@shimadzu.co.jp (Y. Hashi), jmlin@mail.rcees.ac.cn (J.-M. Lin).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.10.022

mine pesticides. However, the combination extraction procedure/determination technique has not been reported yet.

The purpose of this study was to establish a dispersive-SPE method for the simultaneous determination of carbamates and OPPs in fruits and vegetables by LC–MS with a single quadrupole instead of the tripe one. Method validation was presented in terms of recovery and precision, and then it was applied to monitor real samples from local market.

2. Experimental

2.1. Chemicals and reagents

The HPLC grade-water was obtained by purification of de-ionized water through a Milli-Q system (Bedford, USA). Methanol and acetonitrile (LC grade) were purchased from Fisher (New Jersey, USA). Pesticides standards including methiocarb-sulfone, aldicarb, carbaryl, ethiofencarb, isoprocarb, methidathion, azinphos-methyl, baycarb, methiocarb, malathion, pirimiphos-methyl, etrimfos, pyraclofos, phosalone, and formic acid were purchased from Wako (Osaka, Japan). Stock solutions of each pesticide at 1 mg/mL were prepared in methanol and working standard solutions were obtained at various concentrations by dilution of the stock solutions in methanol. These solutions were stored at −25 °C. Analytical grade anhydrous magnesium sulfate ($MgSO_4$) and NaCl were obtained from local reagent supplier in Beijing. Primary secondary amine (PSA) was obtained from Varian (Tokyo, Japan).

2.2. Sample preparation

Ten grams of sample was dissolved in 10 mL acetonitrile and homogenized by vortex for 1 min in a 40 mL centrifuge tube. Four grams of anhydrous $MgSO_4$ and 1 g of NaCl were then added and vortexed immediately for 1 min. It followed by centrifugation at 3000 rpm for 10 min.

One milliliter supernatant aliquot in acetonitrile was transferred into 1.5 mL micro-centrifuge tubes containing 50 mg PSA sorbent and 100 mg anhydrous $MgSO_4$. The vial was tightly sealed and vortexed for 1 min, and then centrifuged for 5 min at 5000 rpm to separate solution from solid. The solution was transferred into the 1.5 mL sample vial and then placed in the autosampler and analyzed by LC–MS.

2.3. Liquid chromatography–mass spectrometry

A Shimadzu LC/MS 2010A system was employed. It consisted of two LC-10ADvp pumps, a DGU-14A degasser, a SIL-HTc autosampler with volume injection set at 10 μL, and a single quadrupole MS. Data acquisition and processing were performed with the LC–MS solution Ver 3.0 Workstation.

The chromatographic separation was performed on a Shim-Pack VP-ODS (150 mm × 2.0 mm i.d., 5 μm). The mobile phase was methanol–water (containing 0.2% formic acid) at total flow-rate of 0.2 mL/min. LC gradient program: 0–25 min, methanol concentration from 20% to 90%; 25–30 min, methanol concentration holds at 90%; 30–40 min, methanol concentration holds at 20%.

Operating condition of the ESI interface in positive ionization mode: CDL temperature, 250 °C, block heater, 200 °C; nebulizer gas (N_2), 1.5 L/min, drying gas (N_2), 0.04 MPa; detector voltage, 1.5 kV; Probe voltage, 4.5 kV.

Selected-ion monitoring (SIM) of the most abundant ions of each compound was used for quantification. However, when two compounds gave similar transitions, another product ion was selected. Therefore, protonated molecular ion (MH^+) was firstly chosen as the precursor ion. In some cases, when the intensity of the molecular ion was too low, the sodium or water adduct ion were chosen as precursor ions such as methiocarb-sulfone, phosalone, and so on.

2.4. Validation study

Linear dynamic range, precision, recovery, selectivity, and uncertainty for the analytical methodology were evaluated. Linearity was determined by calibration curves created with concentrations of 0.01, 0.02, 0.05, 0.10, 0.20, and 0.50 μg/mL by mixture standard solutions. For sample matrix testing, tomato, apple, cabbage, and carrot obtained from supermarket were spiked and tested for recovery, precision at 0.05 mg/kg for each pesticide with five replicates. Before spiked testing, the blank samples were analyzed. If contaminated, the recoveries were calculated by subtraction of blank samples.

3. Results and discussion

3.1. Optimized LC–MS method

Flow-injection testing of individual standard solutions was performed to select ion source. The results demonstrated higher responses in positive mode than in negative mode for both ESI and APCI. In addition, the signal responses were 10–20 times higher by ESI than APCI for all tested pesticides. Therefore, ESI in positive mode was selected for next experiment.

To improve the chromatographic resolution and ionization efficiency of MS, analytical conditions such as LC gradient program, mobile phase composition, and flow rate of drying-gas were optimized. Chromatographic resolution did not change dramatically and the MS signal for most pesticides decreased by a factor of 5–10 when acetonitrile /water was compared to that of methanol/water [6]. This is most likely due to the fact that acetonitrile is a weaker proton donor than methanol (both in aqueous phase and in gaseous phase as well). Methanol was suitable for obtaining high intensity of all carbamates since it is liable to provide hydrogen to the radical ion of carbamates [12]. For optimal LC separation, the more formic acid that was added to the mobile phase, the better resolution obtained for pesticides especially for those solute pairs such as pirimiphos-methyl and etrimfos which were difficult to be separated. The addition of formic acid increased the signal of MH^+ ions, however, the signal-to-noise ratio (S/N) of analytes decreased since the baseline noise increased when formic acid was over 0.2% (v/v) in the mobile phase. Regarding MS sensitivity and optimal

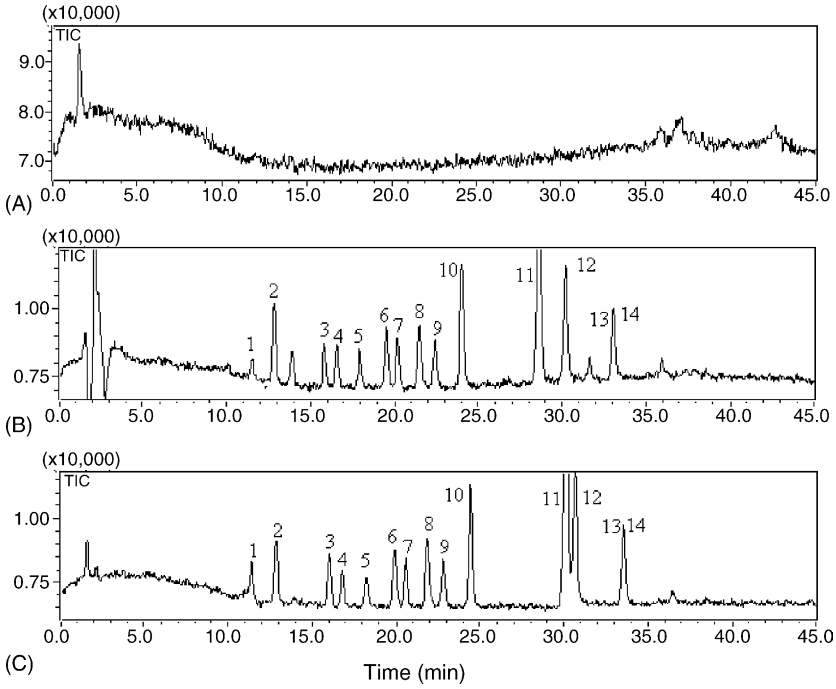

Fig. 1. The chromatograms of pesticides mixture (A) unspiked tomato, (B) spiked tomato (0.05 mg/kg), and (C) standard solution (0.05 μg/mL). (1) Methiocarb-sulfone; (2) aldicarb; (3) carbaryl; (4) ethiofencarb; (5) isoprocarb; (6) methidathion; (7) azinphos-methyl; (8) baycarb; (9) methiocarb; (10) malathion; (11) pirimiphos-methyl; (12) etrimfos; (13) pyraclofos; (14) phosalone.

mobile phase composition for separation, a mixture of methanol and water with the addition of 0.2% formic acid was chosen as eluting solvent. In addition, the flow-rate of drying-gas also played an important role in MS sensitivity. Perfect sensitivity appeared at flow-rate of 0.04 MPa.

After optimization, limits of detection (LODs) were obtained by injection of the standard mixture and calculated with S/N ≥ 3 in SIM mode. Pesticides can be detected at the level of 0.5–10 ng/mL depending on the type of the analytes, which could meet requirements of residue analysis.

3.2. Validation of the method

Calibration curves were established through the range of 0.01–0.5 μg/mL with correlation coefficients from 0.9950 to 0.9999. For real samples such as tomato, credible determination of the pesticide studied were achieved due to the lack of interfering peaks and the low background noise as shown in blank sample (Fig. 1(A)). Chromatograms obtained from LC–MS analysis tomato spiked with 0.05 mg/kg and 0.05 μg/mL standard solution are, respectively, illustrated in Fig. 1(B and C). Compared with the two chromatograms, it is easy to observe an obvious trend that pirimiphos-methyl and etrimfos were separated completely in tomato sample, while only partial separation was achieved by injection of the standard solution. It is thought that pirimiphos-methyl was protonated in the acid tomato matrix which resulted in decreasing the retention on the column. For other types of samples matrices, there is some concern that target pesticides co-eluted with other components at about the same retention time originating from the matrix itself. As a result, with the aid of selection ion chromatogram, co-chromatography of each pesticide enabled the selective and positive identification of peaks of interest. No interfering peaks from endogenous compounds of matrices were found.

Due to the fact that MRLs of most carbamates and OPPs pesticides are over or equal 0.05 mg/kg as shown in Table 1, the precision and accuracy of the above-mentioned method are validated by four samples spiked with 0.05 mg/kg (Table 1). The low recoveries of pirimiphos-methyl may have resulted from the hydrophilic structure which led to considerable solubility in water phase. Methiocarb-sulfone and aldicab showed relatively low recoveries and high RSDs. The reasons could come from two asides. One is that these compounds are liable to degrade during the extraction process [13]; on the other hand, many polar compounds from the matrices exhibited weak retention on column, resulting in the suppression to some pesticides having similar retention behavior. As reported by Jansson et al. [6], the matrix effect is very compound-dependent, probably due to co-eluting matrix components which might interact with the target pesticide within the ionization interface. In addition, the high mean recovery of azinphos-methyl and phosalone could be partly explained by the lower ionization efficiency of the compounds containing an acryl group in working solutions of pure methanol than those ionized from the matrices containing water. These effects demonstrate a different matrix affinity for pesticides as suppression and enhancement for one specific combination of pesticide and matrix. On the whole, the recoveries and RSDs were not influenced adversely by the kind of sample, and the method could serve as a quantitative method to identify and determine the pesticides in vegetables and fruits with reliable results at MRLs.

Table 1
Recovery and RSD of the pesticides in different samples spiked with 0.05 mg/kg ($n = 5$) and maximum residue limits (MRLs) established by Japan (a) and EU (b)

Pesticides	Cabbage		Tomato		Carrot		Apple		MRLs (mg/kg)	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Values	Matrixes
Methiocarb-sulfone	72	2.8	100	5.5	73	6.2	87.0	5.7	0.05[a]	Lettuce
Aldicarb	91	5.1	89	3.3	88	2.8	93	2.4	0.05[a]	Grape
Carbaryl	90	3.3	101	2.4	101	2.4	120	6.5	0.10[a]	Potato
Ethiofencarb	89	2.1	103	1.9	97	1.7	113	3.9	0.50[a]	Potato
Isoprocarb	86	4.0	79	4.7	85	1.7	103	5.9	0.05[b]	Pear
Methidathion	81	1.4	109	2.2	94	2.7	115	1.5	0.30[b]	Pear
Azinphos-methyl	105	0.3	116	1.7	95	1.7	120	2.0	0.50[b]	Apple
Baycarb	90	3.5	98	2.4	93	2.9	104	2.2	0.30[a]	Peach
Methiocarb	102	2.3	104	1.2	100	1.4	117	4.3	0.05[a]	Cabbage
Malathion	90	2.6	99	3.0	94	2.6	107	3.2	0.50[b]	Apple
Pirimiphos-methyl	68	0.8	58	0.3	58	1.0	71	1.4	0.05[b]	Apple
Etrimfos	93	1.9	99	2.5	98	1.0	114	2.2	0.05[a]	Cauliflower
Pyraclofos	81	1.9	94	1.2	96	1.8	112	4.2	0.05[a]	Potato
Phosalone	110	2.3	111	3.6	86	3.4	119	3.3	2.00[b]	Apple

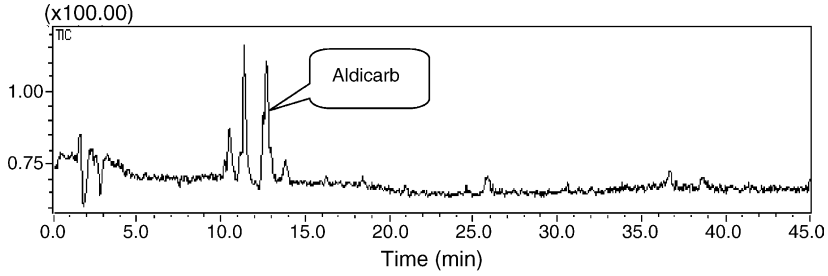

Fig. 2. The typical chromatogram of peach sample.

3.3. Application to real samples

Twenty-five representative samples were collected from local markets including root vegetables (carrot and potato), leafy vegetables (lettuce, cabbage, and spinach), bulb vegetables (onion, pumpkin-squash, and eggplant), fruit vegetables (cucumber and tomato), bean vegetables (kidney bean-legume), and prome fruits (apple, melon, and peach). In those fruits and vegetables above, the pesticides studied were usually monitored by Japan or EU that established the corresponding MRLs as shown in Table 1. Those samples were determined by method established above, and the distribution and concentration of main pesticide residues were shown in Table 2. Some pesticides were also detected in one or two samples at different levels (e.g. 12.7 μg/kg methidathion in one potato); others pesticides (carbaryl, baycarb, methiocarb, pyraclofos, and etrimfos) were not found in any samples. The concentrations found in the samples except for two peaches were always lower than MRLs (see Table 1). Representative chromatogram of peach sample is shown in Fig. 2. On the whole, 70% of samples contained one or more pesticide residues. More than 30% of samples contained multi-residues. In the worst case, there were 10 different pesticides found in a potato sample, but all concentrations found were below MRLs. Obviously, root and leafy vegetables are more susceptible to contamination compared to other samples. Azinphos-methyl and malathion widely existed in almost all types of fruits and vegetables, which indicated these two pesticides are often used. Other pesticides existed in only one or two types of fruits and vegetables, which may be explained by specific use of pesticide. For example, aldicarb residues surpassed the MRLs in two peaches, while it was not found in other samples.

Table 2
Distribution and amount of main pesticides residues in all kinds of fruits and vegetables

Residues	Root (%)	Leafy (%)	Fruit (%)	Prome (%)	Bulb (%)	Total (%)	Amount (μg/kg)
Azinphos-methyl	60	60	20	20	20	36	6.02–73.9
Malathion	40	16	–	20	20	36	2.13–223
Phosalone	60	–	–	–	–	28	0.52–88.6
Pirimiphos-methyl	40	–	20	–	–	12	0.54–6.54
Isoprocarb	–	60	–	–	–	12	9.87–24.3
Aldicarb	–	–	–	40	–	8	130–173
Methiocarb-sulfone	20	–	–	–	–	4	23.5

(–) No residues.

4. Conclusions

LC–MS in combination with dispersive-SPE produced better selective and sensitive analysis of carbamate and OPPs pesticides in fruits and vegetables. It showed satisfactory validation results, such as accuracy, precision, and selectivity. For the pesticides studied, the sensitivity of the method developed was good enough to determine reliably at MRLs. It meets the requirement of quickness, simplicity, and economy for routine screening of pesticide residues and monitoring pesticide residues in the fruits and vegetables.

Acknowledgements

The authors gratefully acknowledge financial support of the National Science Fund for Distinguished Young Scholars of China (No. 20125514), National Natural Science Foundation of China (No. 20437020, 50273046), and Major Research Program of Chinese Academy of Sciences (KZCX3-SW-432).

References

[1] A. Caballo-Lopez, M.D.L. Castro, J. Chromatogr. A 998 (2003) 51.
[2] M.J. Taylor, K. Hunter, K.B. Hunter, D. Lindsay, S.L. Bouhellec, J. Chromatogr. A 982 (2002) 225.
[3] J. Klein, L. Alder, J. AOAC Int. 86 (2003) 1015.
[4] K. Granby, J.H. Andersen, H.B. Christensen, Anal. Chim. Acta 520 (2004) 165.
[5] A. Sannino, L. Bolzoni, M. Bandini, J. Chromatogr. A 1036 (2004) 161.
[6] C. Jansson, T. Pihlström, B.-G. Österdahl, K.E. Markides, J. Chromatogr. A 1023 (2004) 93.
[7] M. Anastassiades, S.J. Lehotay, D. Štajnbaher, F.J. Schenck, J. AOAC Int. 86 (2003) 412.
[8] S.J. Lehotay, A.D. Kok, M. Hiemstra, P.V. Bodegraven, J. AOAC Int. 88 (2005) 595.
[9] S.J. Lehotay, K. Mastovská, S.J. Yun, J. AOAC Int. 88 (2005) 630.
[10] S.J. Lehotay, K. Mastovská, A.R. Lightfield, J. AOAC Int. 88 (2005) 615.
[11] A. Posyniak, J. Zmudzki, K. Mitrowska, J. Chromatogr. A 1087 (2005) 259–264.
[12] C. Crescenzi, A.D. Corcia, E. Guerriero, R. Samperi, Environ. Sci. Technol. 31 (1997) 479.
[13] J. Wang, W. Cheung, D. Grant, J. Agric. Food Chem. 53 (2005) 528.